# VOSSIUS & PARTNER

Patentanwälte

**VIA FAX**
**0041-22-733-5428**

Vossius & Partner POB 86 07 67 81634 München Germany

Organisation Mondiale de la
Propriété Intellectuelle
Case postale 18

1211 GENèVE 20
Schweiz

PATENTANWÄLTE
EUROPEAN PATENT ATTORNEYS
EUROPEAN TRADEMARK ATTORNEYS
Dr. VOLKER VOSSIUS, Dipl.-Chem.
(bis 1992: danach in anderer Kanzlei)
Dr. PAUL TAUCHNER, Dipl.-Chem.
Dr. DIETER HEUNEMANN, Dipl.-Phys.
Dr. PETER A. RAUH, Dipl.-Chem.
Dr. GERHARD HERMANN, Dipl.-Phys.
JOSEF SCHMIDT, Dipl.-Ing.
Dr. HANS-RAINER JAENICHEN, Dipl.-Biol.
Dr. ALEXA VON UEXKÜLL, M.Sc.
Dr. RUDOLF WEINBERGER, Dipl.-Chem.
Dr. WOLFGANG BUBLAK, Dipl.-Chem.
AXEL STELLBRINK, Dipl.-Ing.
Dr. JOACHIM WACHENFELD, (Biol.)
Dr. FRIEDERIKE STOLZENBURG, Dipl.-Biol.
RAINER VIKTOR, Dipl.-Ing.
EUROPEAN PATENT ATTORNEYS
Dr. RENATE BARTH, Dipl.-Chem.
Dr. URSULA ENGLBRECHT, Dipl.-Chem.
Dr. PETER EINMAYR, Dipl.-Chem.
RECHTSANWÄLTE
HELGA TREMMEL
Dr. JOHANN PITZ
BARBARA GUGGENMOS, Dipl.-Chem.
Dr. THURE SCHUBERT
SIMONE SCHÄFER

SIEBERTSTRASSE 4
81675 MÜNCHEN
GERMANY
TEL.: +49-89-41 30 40
FAX: +49-89-41 30 41 11 (G3/G4)
+49-89-41 30 44 00 (G3)
(Marken - Trademarks)
E-MAIL: info@vossiusandpartner.com
HOMEPAGE: www.vossiusandpartner.com

# URGENT

PCT-Patent Application
No. PCT/EP00/08570
EPIDAUROS BIOTECHNOLOGIE AG, et al.
Our Ref.: D 2145 PCT/1

January 17, 2002
Jae/cg

Dear

We have now noticed that the address of the inventors WOJNOWSKI, Leszek and EISELT, Regina have been mixed up on the coversheet of the WO-Publication.

Therefore, we herewith request that the address be corrected as follows ( and mention in the chapter II demand):

WOJNOWSKI, Leszek
Ebenauer Str. 9

80637 München/DE

EISELT, Regina
Albert-von-Iring-Str. 1

82547 Eurasburg/DE

We appreciate receiving the PCT form IB 306 as soon as possible, preferably by facsimile, since the term for entering the national phase expires on March 10, 2002.

Very truly yours,

Dr. Hans-Rainer Jaenichen

Express Mail Label
No. EV 045436952 US

# VOSSIUS & PARTNER

Patentanwälte

Vossius & Partner POB 86 07 67 81634 München Germany

To the
European Patent Office
Munich

PATENTANWÄLTE
EUROPEAN PATENT ATTORNEYS
EUROPEAN TRADEMARK ATTORNEYS
Dr. VOLKER VOSSIUS, Dipl.-Chem.
(bis 1992; danach in anderer Kanzlei)
Dr. PAUL TAUCHNER, Dipl.-Chem.
Dr. DIETER HEUNEMANN, Dipl.-Phys.
Dr. PETER A. RAUH, Dipl.-Chem.
Dr. GERHARD HERMANN, Dipl.-Phys.
JOSEF SCHMIDT, Dipl.-Ing.
Dr. HANS-RAINER JAENICHEN, Dipl.-Biol.
Dr. ALEXA VON UEXKÜLL, M.Sc.
Dr. RUDOLF WEINBERGER, Dipl.-Chem.
Dr. WOLFGANG BUBLAK, Dipl.-Chem.
AXEL STELLBRINK, Dipl.-Ing.
Dr. JOACHIM WACHENFELD, (Biol.)
Dr. FRIEDERIKE STOLZENBURG, Dipl.-Biol.
RAINER VIKTOR, Dipl.-Ing.
EUROPEAN PATENT ATTORNEYS
Dr. RENATE BARTH, Dipl.-Chem.
Dr. URSULA ENGLBRECHT, Dipl.-Chem.
Dr. PETER EINMAYR, Dipl.-Chem.
RECHTSANWÄLTE
HELGA TREMMEL
Dr. JOHANN PITZ
BARBARA GUGGENMOS, Dipl.-Chem.
Dr. THURE SCHUBERT
SIMONE SCHÄFER

SIEBERTSTRASSE 4
81675 MÜNCHEN
GERMANY
TEL.: +49-89-41 30 40
FAX: +49-89-41 30 41 11 (G3/G4)
+49-89-41 30 44 00 (G3)
(Marken - Trademarks)
E-MAIL: info@vossiusandpartner.com
HOMEPAGE: www.vossiusandpartner.com

November 28, 2001
Jae/AD/mb

PCT/EP00/08570
EPIDAUROS BIOTECHNOLOGIE AG et al.
Our Ref.: D2145 PCT/1

This is in response to the Written Opinion issued on August 7, 2001 by the EPO as IPEA in the above identified case.

Enclosed please find new claims 1 to 40.

## 1. Amendments to the claims

1.1 **New claims 1 to 12** correspond to originally filed claims 1 to 12.

1.2 **New claims 13 to 29** correspond to originally filed claims 14 to 30.

1.3 **New claim 30** has been incorporated. Said claim is supported by the description on page 26, lines 23 to 27.

1.4 **New claims 31 to 40** correspond to originally filed claims 32 to 41.

1.5 Originally filed claims 13, 42 and 43 have been deleted from the new set of claims.

## 2. Clarity (Article 6 PCT)

### 2.1 Claim 3

In section VIII.a the Examiner took the position that claim 3 currently on file lacked clarity since the gene would be characterized by the effect which should be achieved.

However, claim 3 is dependent on claim 1. The altered expression of the variant CYP3A4 or CYP3A7 gene is a technical feature which further characterizes the polynucleotide of claim 1 or 2. Thus, the subject matter of claim 3 is also defined by the polynucleotides of claim 1 or 2 resulting in altered expression of the variant CYP3A4 or CYP3A7 gene. Therefore, claim 3 is clear.

### 2.2 Claims 15 to 17

In section VIII.b the Examiner took the position that the claimed transgenic animal may naturally contain one of the polynucleotides of any one of claims 1 to 3.

However, claims 15 to 17 relate to a non-human transgenic animal. As is evident from the sequence listing and the description on page 10, lines 6 to 9, the polynucleotides and variants thereof comprised by claims 1 to 3 are derived from human. A non-human transgenic animal does by no means naturally comprise "human" polynucleotides in its genome. Thus, the argument set forth by the Examiner is unfounded. Therefore, claims 15 to 17 are clear.

## 3. Sufficiency of disclosure (Article 5 PCT)

The Examiner took the position that the association between polymorphisms and a disease lacked support by the application. Thus, the corresponding claims would not be sufficiently disclosed.

However, on page 13, lines 19 to 22 it is disclosed that the polynucleotides of the invention caused altered expression of CYP3A4. A subject comprising

those polynucleotides has an altered CYP3A4 activity which, as described on page 2, lines 28 to 30, causes cancer, such as liver cancer.
Thus, the association with an altered activity of CYP3A4 has been very well known in the art. In accordance with this invention, applicant could convincingly demonstrate by the well accepted in vitro method assays described in the examples, that the polymorphism polynucleotides are inter alia a cause of cancer.

## 4. Novelty (Article 33(2) PCT)

### 4.1 Previous claim 31 and 32 and new claims 31 and 32

In section V.a the Examiner set forth that claims 31 and 32 lacked novelty. Said claims would merely relate to the treatment of a known disorder by known drugs.

As set forth under item 1.3, new claim 31 is now dependent on claim 30. Novelty of claim 30 has been acknowledged by the Examiner. As a consequence, new claim 31 and new claim 32 which is dependent thereon are also novel.

### 4.2 Previous claims 42 to 43

In section V.a the Examiner took the position that claims 42 and 43 also lacked novelty for the same reason as set forth for previous claims 31 and 32.

Previous claims 42 and 43 have been deleted. Thus, the objection does not apply for the new set of claims.

## 5. Inventive step (Article 33(3) PCT) of new and previous claims 1 to 12, 14 to 30 and 35 to 41

The Examiner argues in section V.d that the above claims as far as CYP3A4 is concerned lacked inventive step since the implications of the altered CYP3A4 activity disclosed in the application for a subject carrying said altered CYP3A4 would not have been investigated.

We disagree.

The technical problem underlying the present invention is to provide means and methods for diagnosing the efficacy of drug based therapies of diseases such as cancer, as described on page 5, lines 25 to 29.

The technical problem is solved by providing the polymorphic polynucleotides disclosed in the present application. On, e.g., page 13, lines 9 to 12 it is disclosed that said polynucleotides result in an altered expression of those CYP3A4 alleles which comprise the said polymorphic polynucleotides.

As set forth by the examiner, applicant could demonstrate the effect of the polymorphic polynucleotides on CYP3A4 activity.
From page 3, lines 4 to 10, it follows that polynucleotides resulting in an altered CYP3A4 activity are the cause of an inefficient drug based therapy, a fact which is well known in the art. Therapeutic drugs in the sense of the present invention are also disclosed, see page 2, lines 24 to 28.
Thus, the in vitro model for CYP3A4 activity demonstrates unambiguously that a CYP3A4 polypeptide encoded by a polymorphic polynucleotide of the invention has an altered activity. It follows form the above that it can be inevitably concluded from said result that a subject comprising said polymorphic polynucleotide will also have altered CYP3A4 activity and can, therefore, not be efficiently treated by the standard drug based therapies for cancer.
This relation is also demonstrated in the table of Annex A.

## 6. Industrial applicability (Article 33(4) PCT) of new and previous claims 28, 29, 31, 42 and 43

In section V 3 the Examiner set forth that the aforementioned claims may be regarded as lacking industrial applicability in some of the PCT member states.

Said claims may be amended when the international application enters the national and/or regional phases.

## 7. Request

With the above explanations and the claim amendments applicant has met the PCT requirements.

Therefore, it is requested that a more favourable IPER be issued.

Renate Barth

Dr. Renate Barth
European Patent Attorney

**Enclosures:**
New claims 1 to 40, in triplicate
Annex A

# Claims

1. A polynucleotide selected from the group consisting of:

   (a) a polynucleotide having the nucleic acid sequence of SEQ ID NO: 54, 55, 58, 59, 62, 63, 66, 67, 70, 71, 74, 75, 78, 79, 82, 83, 86, 87, 90, 91, 94, 95, 98, 99, 102, 103, 106, 107, 110, 111, 118, 119, 122, 123, 126, 127, 128, 134, 138, 144, 146, 148, 150, 151, 152, 153, 154, 156, 157, 159, 161, 162, 163, 164 or 171;

   (b) a polynucleotide encoding a polypeptide having the amino acid sequence of any one of SEQ ID NO: 129, 135, 139, 145, 147, 155, 158, 160 or 172;

   (c) a polynucleotide encoding a CYP3A4 or CYP3A7 polypeptide, wherein said polynucleotide is having at a position corresponding to any one of position 6004, 13908, 14292, 14304, 14323, 14329, 14357, 15753, 20230, 21867, 21868, 21896, 22026, 22041, 23081, 23172, 25925 or 25958 of the CYP3A4 gene (Accession No: AF280107, whereby the nucleotide A of the first ATG encoding the CYP3A4 protein has been taken as position 1) or at a position corresponding to position 1229 of the CYP3A7 (Accession No: gi4503232) a nucleotide exchange, a nucleotide deletion, an additional nucleotide or an additional nucleotide and a nucleotide exchange, wherein said nucleotide deletion at a position corresponding to position 23172 is not resulting in an M to T amino acid substitutuion or is not a T to C nucleotide exchange;

   (d) a polynucleotide encoding an CYP3A4 or CYP3A7 polypeptide, wherein said polynucleotide is having at a position corresponding to any one of position 6004, 13908, 14292, 20230 or 21868 of the CYP3A4 gene (Accession No: AF280107, whereby the nucleotide A of the first ATG encoding the CYP3A4 protein has been taken as position 1) an A, at a position corresponding to any one of position 14323, 14329, 21867, 21896, 22026, 22041, 23081 or 25925 of the CYP3A4 gene (Accession No: AF280107, whereby the nucleotide A of the first ATG encoding the CYP3A4 protein has been taken as

position 1) a T, at a position corresponding to any one of position 14357, 15753 or 25958 of the CYP3A4 gene (Accession No: AF280107, whereby the nucleotide A of the first ATG encoding the CYP3A4 protein has been taken as position 1) a G, at a position corresponding to any one of position 14304 of the CYP3A4 gene (Accession No: AF280107, whereby the nucleotide A of the first ATG encoding the CYP3A4 protein has been taken as position 1) a C or at a position corresponding to position 1229 of the CYP3A7 gene (Accession No: gi4503232) a G;

(e) a polynucleotide encoding an CYP3A4 polypeptide, wherein said polypeptide comprises an amino acid substitution at any one of position 56, 130, 170, 174, 363, 373, 416 or 445 of the CYP3A4 polypeptide (Accession No: AF280107), wherein said substitution at a position corresponding to position 445 is not M to T; and

(f) a polynucleotide encoding an CYP3A4 or CYP3A7 polypeptide, wherein said polypeptide comprises an amino acid substitution of G to D at position 56, R to Q at position 130, V to I at position 170, D to H at position 174, T to M at position 363, L to F at position 373 or P to L at position 416 of the CYP3A4 polypeptide (Accession No: AF280107) or T to R at position 409 of the CYP3A7 polypeptide (Accession No: gi4503232).

2. The polynucleotide of claim 1, wherein said polynucleotide encodes a variant CYP3A4 or CYP3A7 protein or fragment thereof.

3. The polynucleotide of claim 1 or 2, wherein the nucleotide deletion, addition and/or substitution result in altered expression of the variant CYP3A4 or CYP3A7 gene compared to the corresponding wild type gene.

4. A vector comprising the polynucleotide of any one of claims 1 to 3.

5. The vector of claim 4, wherein the polynucleotide is operatively linked to expression control sequences allowing expression in prokaryotic or eukaryotic cells.

6. A host cell genetically engineered with the polynucleotide of any one of claims 1 to 3 or the vector of claim 4 or 5.

7. A method for producing a molecular variant CYP3A4 or CYP3A7 protein or fragment thereof comprising
   (a) culturing the host cell of claim 6; and
   (b) recovering said protein or fragment from the culture.

8. A method for producing cells capable of expressing a molecular variant CYP3A4 or CYP3A7 gene comprising genetically engineering cells with the polynucleotide of any one of claims 1 to 3 or the vector of claim 4 or 5.

9. A CYP3A4 or CYP3A7 protein or fragment thereof encoded by the polynucleotide of any one of claims 1 to 3 or obtainable by the method of claim 7 or from cells produced by the method of claim 8.

10. An antibody which binds specifically to the protein of claim 9.

11. The antibody of claim 10 which specifically recognizes an epitope containing one or more amino acid substitution(s) as defined in any one of claims 1 to 3.

12. A nucleic acid molecule complementary to a polynucleotide of any one of claims 1 to 3.

13. A vector comprising the nucleic acid molecule of claim 12.

14. A transgenic non-human animal comprising at least one polynucleotide of any one of claims 1 to 3 or the vector of claim 4 or 5.

15. The transgenic non-human animal of claim 14 further comprising at least one inactivated wild type allele of the CYP3A4 or CYP3A7 gene.

16. The transgenic non-human animal of claim 14 or 15, which is a mouse or a rat.

17. A method of identifying and obtaining a CYP3A4 or CYP3A7 inhibitor capable of modulating the activity of a molecular variant of the CYP3A4 or CYP3A7 gene or its gene product comprising the steps of
    (a) contacting the protein of claim 9 or a cell expressing a molecular variant CYP3A4 or CYP3A7 gene comprising a polynucleotide of any one of claims 1 to 3 in the presence of components capable of providing a detectable signal in response to drug metabolization, with a compound to be screened under conditions to permit CYP3A4- or CYP3A7-mediated drug metabolization, and
    (b) detecting the presence or absence of a signal or increase of a signal generated from the drug metabolization, wherein the presence or increase of the signal is indicative for a putative inhibitor.

18. The method of claim 17 wherein said cell is a cell of claim 6, obtained by the method of claim 8 or is comprised in the transgenic non-human animal of any one of claims 14 to 16.

19. A method of identifying and obtaining an CYP3A4 or CYP3A7 inhibitor capable of modulating the activity of a molecular variant of the CYP3A4 or CYP3A7 gene or its gene product comprising the steps of
    (a) contacting the protein of claim 9 with a first molecule known to be bound by CYP3A4 or CYP3A7 protein to form a first complex of said protein and said first molecule;
    (b) contacting said first complex with a compound to be screened; and
    (c) measuring whether said compound displaces said first molecule from said first complex.

20. The method of claim 19, wherein said measuring step comprises measuring the formation of a second complex of said protein and said compound.

21. The method of claim 19 or 20, wherein said measuring step comprises measuring the amount of said first molecule that is not bound to said protein.

22. The method of any one of claim 19 to 21 wherein said first molecule is nifedipine, rifampicine or corticosterone.

23. The method of any one of claims 19 to 22 wherein said first molecule is labeled.

24. A method of diagnosing a disorder related to the presence of a molecular variant of the CYP3A4 or CYP3A7 gene or susceptibility to such a disorder comprising
    (a) determining the presence of a polynucleotide of any one of claim 1 to 3 in a sample from a subject; and/or
    (b) determining the presence of a protein of claim 9.

25. The method of claim 24, wherein said disorder is cancer.

26. The method of claim 24 or 25 comprising PCR, ligase chain reaction, restriction digestion, direct sequencing, nucleic acid amplification techniques, hybridization techniques or immunoassays.

27. The method of any one of claims 24 to 26, further comprising administering to a subject a medicament to abolish or alleviate said disorder.

28. The method of any one of claims 24 to 27, further comprising introducing
    (i) a functional and expressible wild type CYP3A4 or CYP3A7 gene or

(ii) a nucleotide acid molecule of claim 12 or the vector of claim 14 into cells.

29. A method for the production of a pharmaceutical composition comprising the steps of the method of any one of claims 17 to 23; and

(c) synthesizing and/or formulating the compound identified and obtained in step (b) or a derivative thereof in a pharmaceutically acceptable form.

30. The method claim 29, wherein said compound or derivative thereof is a drug or prodrug in a form suitable for therapeutic application and preventing or ameliorating the disorder of the subject diagnosed in the method of claim 24 or 25.

31. The method of claim 29 or 30 wherein said compound drug or prodrug is a derivative of a medicament as defined in claim 28.

32. An inhibitor identified or obtainable by the method of any one of claims 17 to 23.

33. The inhibitor of claim 32 which binds specifically to the protein of claim 9.

34. Use of an oligo- or polynucleotide for the detection of a polynucleotide of any one of claims 1 to 3 and/or for genotyping of individual CYP3A4 or CYP3A7 alleles.

35. The use of claim 34 wherein said polynucleotide is a polynucleotide of any one of claims 1 to 3 or a nucleic acid molecule of claim 12.

36. The use of claim 34 wherein said oligonucleotide is about 15 to 50 nucleotides in length and comprises the nucleotide sequence of any one of SEQ ID NOS: 1 to 127, 140 or 141 or a complementary sequence.

37. A primer or probe consisting of an oligonucleotide as defined in claim 36.

38. Use of an antibody or a substance capable of binding specifically to the gene product of an CYP3A4 or CYP3A7 gene for the detection of the protein of claim 9, the expression of a molecular variant CYP3A4 or CYP3A7 gene comprising a polynucleotide of any one of claims 1 to 3 and/or for distinguishing CYP3A4 alleles comprising a polynucleotide of any one of claims 1 to 3.

39. A composition comprising the polynucleotide of any one of claims 1 to 3, the vector of claim 4 or 5, the host cell of claim 6 or obtained by the method of claim 8, the protein of claim 9, the antibody of claim 10 or 11, the nucleic acid molecule of claim 12, the vector of claim 13, the inhibitor of claim 32 or the primer or probe of claim 37.

40. The composition of claim 39 which is a diagnostic or a pharmaceutical composition.